Remarks

Applicants would like to thank Examiner Lin and Primary Examiner Borin for the courtesy extended to the Applicant, Dr. David Rimm and Applicant's representatives in the interview on July 25, 2006. Applicants have received the Interview Summary Record, mailed August 2, 2006 which generally reflects Applicants' understanding of the interview. Applicants note that it was also discussed and suggested that wording the claims to reflect the step achieved by the algorithms disclosed rather than reciting the algorithm per se would be acceptable and to recite cells included within a tissue sample would be consistent with the previously presented claims. As discussed during the interview, important differentiating aspects of the invention as reflected in the claims set forth above include the following:

a. use of at least three stains at least two of which are used to define subcellular compartments;

b. use of an upright or inverted optical microscope rather than a confocal microscope;

c. examination of cells within a tissue sample;

d. assigning pixel locations to alternative subcellular compartments;

e. reiteratively analyzing pixel locations based upon relative intensity values of the stains; and

f. effecting both localization and quantitation of a biomarker of interest.

Claims 9, 10, 39 and 47-59 are currently pending. Claims 1-8, 11-38, 40-46 have been canceled without disclaimer or prejudice to applicant's right to pursue such claims in the future and claims 9, 10 and 39 are amended herein. New claims 47-59 are presented to expedite prosecution by focusing on what Applicants regard as an important embodiment of their invention. All amended and newly presented claims are fully supported by the instant specification as originally filed as discussed further below. No new matter has been introduced. Independent claim 47, from which claims 48-58 depend, and independent claim 59 are directed

to a computer-implemented method for localizing and quantitating a particular biomarker within subcellular compartments present in individual cells of interest contained in a tissue sample.

Support for independent claims 47 and 59 can be found, for example, in Example 1 (pages 27-29), which describes localization and quantitation of biomarkers (estrogen receptor (ER), HER2/neu or beta-catenin) within a first subcellular compartment, (i.e. the cell nucleus stained with DAPI) and/or a second subcellular compartment (i.e. the cellular membrane stained with anti-alpha catenin) by immunohistochemistry staining (page 27 line 21 to page 28 line 10), obtaining high resolution images (page 28 lines 11-17) and analyzing the images (page 28 lines 17-31) . Additional support for claims 47 and 59 can be found, for example, on page 5, lines 3-12 and page 10, line 21 to page 13, line 2, for example localization of subcellular compartments in cells (see page 5, line 5), cells of interest within a tissue section (page 7 lines 7-8), a computer implemented technique which measures the relative intensities of images derived from compartment–specific stains on a pixel-by-pixel basis (page 5 lines 6-7) and summing the total intensity values (page 13 lines 4-9). Further support for claim 59, for example, the established degree of accuracy can be found at page 5, lines 10-11 and page 11, lines 3-4; assignment ratios are described at page 11, lines 5-11, weighted ratios at page 12, lines 7-17. Support for claim 48 can be found, for example on page 13, line 10-18. Support for claim 49 can be found, for example on page 11, lines 10-11. Support for claim 50 can be found, for example, in Example 1 (page 27, lines 15-18). Support for dependent claim 51 can be found, for example in Example 1 (page 28, lines 18-19). Support for claim 52 can be found, for example in Figure 1, as described at page 5, lines 13-21 and in Example 1. Support for claim 53 can be found in Example 1 (page 27, line 12). Support for claim 54 can be found, for example at page 5, line 17 and 19, page 23, line 14, and page 28, line 1. Support for claim 55 can be found, for example on page 20, line 18 and in Example 1. Support for claim 56 can be found, for example on page 22, line 22 and in Example 1. Support for claim 57 can be found, for example on page 9, line 10 to page 10, line 20 and at page 13, line 19. Support for claim 58 can be found, for example on page 7, line 20 to page 9, line 9.

Claim Rejections

Rejection of Claims 44-46 Under 35 U.S.C. § 112, 1st Paragraph

The examiner has rejected claims 44-46 under 35 U.S.C. § 112, 1st paragraph. This rejection is moot as claims 44-46 have been canceled.

Rejection of Claims 7-29, 39, 40, 42, 44-46 Under 35 U.S.C. § 101

The Examiner has rejected claims 7-29, 39, 40, 42, 44-46 under 35 U.S.C. §101 as drawn to non-statutory subject matter. This rejection is moot as claims 7-8, 11-29, 40, 42, 44-46 have been canceled and claims 9-10 and 39 have been amended to depend on new claim 47. This rejection is not applicable to the new claims which include a physical step leading to a useful, tangible and concrete result.

Rejection of Claims 7, 9-19, 23-29, 39, 40 and 42 Under 35 U.S.C. § 102 and Rejection of Claim 8 Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 7, 9-19, 23-29, 39, 40 and 42 under 35 U.S.C. 102(e)(1) as being anticipated by Harris et al. (US 2003/0036855) and claim 8 under 35 U.S.C. 103 (a) as obvious over Harris et al. in view of Rigaut et al. These rejections are moot as claims 7, 8, 11-19, 23-29, 40 and 42 have been canceled and claims 9-10 and 39 have been amended to depend on new claim 47. Furthermore, Harris et al. does not teach or suggest new independent claims 47 and 59 and therefore do not teach claims 9-10, 39 and 48-58 which depend therefrom. Harris teaches a biological assay using a line-scan confocal microscope imaging system. One embodiment is an assay of two fluorescently labeled species and co-localization of the two stains in a cell or sub-cellular structure. To accomplish this, Harris generates a binary bitmap from the image of one of the stains, (i.e. a mask) and then measures the intensity of the second stain in the bitmapped pixels. In contrast, Applicant's invention provides a method of analyzing fluorescently labeled tissue specimens with a conventional optical microscope which achieves a resolution thought unachievable without resorting to a costly confocal microscope. As claimed, Applicants use the intensity values (not binary) of two stains relative to each other, to accurately define two subcellular compartments and then measure the intensity value of a third stain labeled

biomarker in such subcellular compartments. Additional differentiating aspects of Applicants' now claimed method are listed above.

Rigaut et al., which has been cited as teaching "a method wherein images are taken at different depths and used to manipulate the image pixel intensities (i.e. features) (page 512, right column)" does not make up for the deficiencies of Harris et al.. Rigaut teaches three dimensional imaging of DNA content in tissue blocks using a confocal microscope. Rigaut does not localize or quantitate a biomarker in a subcellular compartment.

CONCLUSION

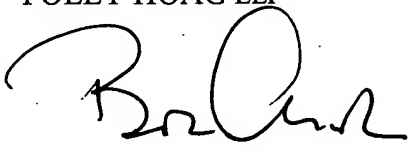
For the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the pending rejections. Applicants maintain that the claims now pending are in condition for allowance, and notification of such is respectfully requested.

The Commissioner is hereby authorized to credit any overpayment or charge any deficiencies to Deposit Account Number **06-1448, Reference YUA-001.01**.

If, for any reason, a telephonic conference with Applicants' undersigned representative would be helpful in expediting prosecution of the instant application, the Examiner is invited to call Applicants' representative at the telephone number provided below.

Respectfully submitted,

FOLEY HOAG LLP



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